

Effect of penicillin and spectinomycin given for urethritis and cervicitis with *Neisseria gonorrhoeae*: high prevalence of penicillin-resistant isolates

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SUMMARY Efficacy of single-dose spectinomycin (TRO: 2 g intramuscularly) was compared with that of aqueous procaine penicillin G (APPG: 4.8×10^6 units) plus 1 g of probenecid for treatment of gonococcal urethritis and cervicitis. Cure rates of the 210 patients who received TRO and 190 patients who received APPG were 97.6% and 91.1%, respectively. MICs of antibiotics were determined using the agar dilution method. Those isolates with MICs of APPG of $< 1.0 \mu\text{g/ml}$ had low failure rates (2.9%), while strains with increased resistance to APPG (MICs $\geq 1.0 \mu\text{g/ml}$) had higher failure rates (24%). Treatment failures seen with TRO were not correlated to isolates with the higher MICs. Clinical results suggest TRO could be given for treatment of genital gonorrhoea in Puerto Rico due to the high prevalence of both chromosomally-mediated penicillin-resistant *Neisseria gonorrhoeae* (20%) and penicillinase-producing *Neisseria gonorrhoeae* (7.5%) strains and the high rate of failure seen with the use of APPG.

The failure of penicillin and spectinomycin regimens in the clinical setting are known to vary according to geographic location.¹⁻³ The reports of significant spatial and prevalence variation in the distribution of penicillinase-producing *Neisseria gonorrhoeae* (PPNG),⁴⁻⁸ chromosomally mediated penicillin-resistant *Neisseria gonorrhoeae* (CMRNG) strains,⁹⁻¹¹ and spectinomycin-resistant isolates¹²⁻¹⁵ are causes for concern among health-care practitioners world-wide. Because of these observations and because of increased travel from the United States, Latin America, and Europe to Puerto Rico, the Latin American Center for Sexually Transmitted Diseases (LACSTD), located in Puerto Rico, decided to compare the efficacy of the penicillin regimen with that of spectinomycin. This study was part of a larger Inter-American project to monitor gonococcal resistance, a research venture generated by the Pan American Health Organization (PAHO) with the participation of the U.S. Centers for Disease Control in Atlanta, Georgia.

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Methodology

Patients

In this study, 533 patients with confirmed genital gonorrhoea by culture were enrolled from among persons visiting LACSTD during the period from 1982 to 1984. Patients were invited to participate if they had signs or symptoms of genital infection and gram-negative intracellular diplococci on smear of the discharge or were contact cases from a culture positive patient. Subjects were not invited to participate if pregnant or if they had a history of allergy to penicillin or spectinomycin, evidence of coexistent syphilis, or had received antibiotics within the previous two weeks. All female and male subjects were between 18 and 54 years of age and gave written and informed consent. Clinical evaluation included a work-up for STD, including a clinical history, VDRL test, gram-stain, KOH and wet mount smears, and pretreatment cultures in modified Thayer-Martin.

Patients were assigned by a randomised table to receive either 4.8 million units of intramuscular aqueous procaine penicillin G (APPG) plus 1 g of oral probenecid or 2 g of intramuscular spectinomycin (Trobicin). Treatment was initiated immediately after oral, genital and anal specimens were obtained for

Table 1 Efficacies of penicillin and spectinomycin in the treatment of gonococcal urethritis or cervicitis

Drug regimen	Number of patients enrolled	Test of cure		Percent of treatment failures
		Positive	Negative	
Aqueous procaine penicillin plus probenecid	190	17	173	8.9
Spectinomycin	210	5	205	2.4
Total number of isolates	400	22	378	5.5

bacterial isolation and identification. Patients with an oral or rectal gonococcal infection, or a negative genital infection were excluded.

Bacterial isolates

We obtained 533 pretreatment isolates of *Neisseria gonorrhoeae* and confirmed their identity by colonial morphology, gram-stain, positive oxidase reaction, and by the coagglutination (Phadebact, Pharmacia Diagnostic, Piscataway, NJ) reaction tests. Identification of clinical isolates was usually completed 48 to 72 hours after specimens were taken. All isolates were tested for beta-lactamase production using the iodometric paper strip test and then frozen in defibrinated rabbit blood at -80°C for further studies.^{16 17}

Susceptibility testing

The minimal inhibitory concentration (MICs) for the *Neisseria gonorrhoeae* isolates were determined using the agar dilution method in which serial two-fold dilutions of the antibiotics were incorporated into Protease Number 3 Agar Base (GIBCO Laboratories, Madison, WI) supplemented with 2% bovine haemoglobin and 1% supplement B (BBL Microbiology Systems, Becton, Dickinson & Co, Cockeysville, MD).¹⁸ The isolates were grown overnight in chocolate agar and then suspended in triplicate soy broth. The reference strains of *Neisseria gonorrhoeae* included for daily quality control of the test were 76-061783, F-28, and F-29 from the Centers for Disease Control in Atlanta, GA. The cell suspension and inoculation of antibiotic containing plates were done as previously described in the literature.^{4 15 18} The MICs were determined as the lowest concentra-

tion permitting the growth on no more than one colony.

Penicillin susceptible *Neisseria gonorrhoeae* isolates were defined as those having a MIC of $\leq 0.06 \mu\text{g/ml}$; intermediately penicillin resistant *Neisseria gonorrhoeae* had MICs in the range $0.06 \mu\text{g/ml}$ to $1.0 \mu\text{g/ml}$; and penicillin resistant *Neisseria gonorrhoeae* were those with MICs $\geq 1.0 \mu\text{g/ml}$.¹⁸ The PPNG and CMRNG isolates were included in the last group because the MICs of these strains were $\geq 1.0 \mu\text{g/ml}$. Spectinomycin susceptible isolates were those with MICs $< 40 \mu\text{g/ml}$ and spectinomycin relatively resistant isolates were those with MICs $\geq 40 \mu\text{g/ml}$.¹⁸

Results

Investigators were unable to evaluate 133 patients either because they did not comply with the test of cure 3 to 10 days after treatment or did have a positive rectal or oral culture (69 were from the spectinomycin group). Thus, 400 of the 533 subjects who had had a positive genital culture for *Neisseria gonorrhoeae* were evaluated. Patients with post-gonococcal urethritis were all treated with oral tetracycline hydrochloride (500 mg 4 times per day for at least 7 days).

Of the subjects that were evaluated, the aqueous procaine penicillin G (APPG) plus probenecid was given to 190 patients (164 men and 26 women) while 210 patients (189 men and 21 women) received spectinomycin. The patients who received spectinomycin had a mean age of 26 years (range, 18–53 years); 134 were single and 72 were married; 186 were heterosexuals and 20 were male homosexuals. The patients in the APPG regimen had a mean age of 26

Table 2 MIC of penicillin and spectinomycin for isolates of *Neisseria gonorrhoeae* from patients with a given drug regimen

Drug regimen	Number tested	Number of isolates			MIC of spectinomycin ($\mu\text{g/ml}$)	
		MIC of penicillin ($\mu\text{g/ml}$)			< 40	≥ 40
		(≤ 0.06)	($0.125-0.50$)	(≥ 1.0)		
Aqueous procaine penicillin G plus probenecid	190	52	84	54	161	29
Spectinomycin	210	59	95	56	177	33
Total number of isolates (%)	400	111 (28)	179 (45)	110 (27)	338 (84)	62 (16)

Table 3 Results of treatment in each drug regimen according to MIC ranges and penicillin resistant isolates

Percentage of treatment failure in each drug regimen	MIC range of penicillin ($\mu\text{g/ml}$)		Penicillin resistant isolates*	
	<1.0	≥ 1.0	PPNGs	CMRNGs
Aqueous procaine penicillin G plus probenecid	2.9% (4/136)	24% (13/54)	36% (5/14)	20% (8/40)
Spectinomycin	MIC range of spectinomycin ($\mu\text{g/ml}$)			
	<40	≥ 40		
	2.8% (5/177)	0% (0/33)	0% (0/16)	5% (2/40)

*There were 110 penicillin-resistant isolates (those with MICs $\geq 1.0 \mu\text{g/ml}$). PPNGs and CMRNGs stand for penicillin-producing *Neisseria gonorrhoeae* and chromosomally mediated-resistant *Neisseria gonorrhoeae* isolates, respectively.

years (range, 18–50 years); 129 were single and 58 were married; 165 were heterosexuals and 22 were male homosexuals. Information of social status and sexual preference was not recorded for seven males. Thus, both groups had similar characteristics. This was consistent with the randomised patient assignment used for this study.

There were no serious adverse reactions or side effects seen in either treatment group. However, post-gonococcal urethritis (defined as 4 or more pus cells per field in a gram-stained smear $\times 1000$ and a negative culture for *Neisseria gonorrhoeae*) was observed in 61 patients (27 spectinomycin; 34 APPG plus probenecid).

The follow-up results are shown in table 1. None of the patients who failed to be cured using either penicillin or spectinomycin admitted re-exposure between visits. All treatment failures with either antibiotic regimen were cured after being retreated with spectinomycin. The penicillin regimen was effective in 91.1% of the patients while the spectinomycin regimen was effective in 97.6%. A chi-square test of the frequencies of cure in each group confirmed that the observed difference was significant ($X(1) = 8.31$, $p \leq 0.01$). Although the sample size of women is small (47) for making conclusions, three of 26 patients treated with penicillin failed to cure and none of 21 treated with spectinomycin failed to cure. These observations among women followed the general trend of entire sample of 400 patients (see table 1).

The number of isolates and treatment failures in

each drug regimen are presented with their corresponding MICs to the study drugs in tables 2 and 3. These data show the relationship of susceptibilities to efficacy. There was no significant relationship of elevated MICs to spectinomycin with failure to respond to therapy.

Among patients receiving the APPG regimen, there was a positive relationship between the number of patients not cured and the number of isolates with the higher MICs. The strains with MICs $\geq 1.0 \mu\text{g/ml}$ had failure rates of 2.9%, while isolates with increased resistance to penicillin, MIC $\geq 1.0 \mu\text{g/ml}$, had failure rates of 24% (see table 3). The failure rates in the two groups were significantly different ($X(1) = 16.56$, $p \leq 0.01$).

A total of 110 isolates of *Neisseria gonorrhoeae* had increased resistance for penicillin (MIC $\geq 1.0 \mu\text{g/ml}$). Eighty of these isolates were CMRNG and 30 were PPNG strains. The distribution of these strains by MICs and results of treatment for each antibiotic regimen are summarised in table 3. In the PPNG strains, 36% of those treated with penicillin failed to respond, while no treatment failures were observed with spectinomycin. In the CMRNG strains, 20% of those treated with penicillin failed to respond, whereas 5% of those treated with spectinomycin failed to respond.

The MIC₅₀ and MIC₉₀ to penicillin and spectinomycin were determined for 533 isolates (table 4). The MIC range and the MIC₅₀ and MIC₉₀ of penicillin and spectinomycin for the 400 isolates from patients with cures are identical. This finding means that the susceptibility pattern of the 400 isolates was similar to the total pool of isolates and similar cure rates will be expected with the 25% dropout patients.

Table 4 MIC₅₀ and MIC₉₀* of *Neisseria gonorrhoeae* isolates† to penicillin and spectinomycin

Antimicrobial agent	MIC range ($\mu\text{g/ml}$)	MIC ₅₀ ($\mu\text{g/ml}$)	MIC ₉₀ ($\mu\text{g/ml}$)
Penicillin	0.004–8.0	0.25	2.0
Spectinomycin	2.5–80	20.0	40.0

*The MIC₅₀ and the MIC₉₀ are the concentrations required to inhibit 50% and 90% of the strains, respectively.

†The MICs of penicillin and spectinomycin were determined for 533 isolates.

Discussion

A high prevalence (16%) of CMRNG strains (MICs $\geq 1.0 \mu\text{g/ml}$) were reported in Puerto Rico in 1981.¹⁸ The number of CMRNG seen is high compared with that seen in the United States^{4,9,10} and in South Africa¹⁹ but similar to other countries.^{1,16} Our results

show a slight increase to 20-5%. Of the 369 non-beta-lactamase producing strains, 72.3% were found to require penicillin MICs of $\leq 1.0 \mu\text{g/ml}$, and they were classified either as penicillin susceptible (MICs $\leq 0.06 \mu\text{g/ml}$) or intermediate resistant strains (MICs $> 0.06 \mu\text{g/ml}$ and $< 1.0 \mu\text{g/ml}$). This percentage is significantly higher than that reported for the United States^{4,6,20-23} but similar to other countries.^{1,16,24}

The incidence of PPNGs from 1981 to 1984 in Puerto Rico were 0.0%, 0.7%, 1.0%, and 8.6%, respectively. The presence of a fair number of PPNG isolates during the study was due to a large outbreak during 1984. Since control efforts are lacking for CMRNG and travel from areas of high prevalence caused periodic outbreaks of PPNG in Puerto Rico, a rapid increase in penicillin-resistant strains can be expected to occur at any time.

The treatment failures observed while using penicillin against strains with MICs $< 1.0 \mu\text{g/ml}$ is very low and comparable to other reports.¹⁻⁴ The positive relationship of penicillin failures with CMRNG strains (MICs $\geq 1.0 \mu\text{g/ml}$) has been previously observed.⁴ The presence of CMRNG strains should be of great concern for countries in the Caribbean and Latin America that still used APPG regimen for gonorrhoea. Therefore, surveillance of these strains should be highly encouraged.¹⁰ In our opinion, a failure rate of greater than 5%, as seen with the APPG plus probenecid regimen in Puerto Rico, does not guarantee effective control of gonorrhoea.

The MIC₅₀ and MIC₉₀ to spectinomycin were higher than in other countries.^{4-6,13,19,20} Perhaps this is due to the frequency of spectinomycin use in Puerto Rico for gonococcal infection. Like others whose work precedes ours, we were unable to relate spectinomycin treatment failures with in vitro spectinomycin susceptibility.¹⁻⁵ The spectinomycin regimen was also effective for PPNG, CMRNG (5% failure rate), and beta-lactamase negative strains, also reported by others.^{4,5,8,11,25}

Factors contributing to treatment failure seen with spectinomycin in this study are still undefined since all isolates with MICs of 40-80 $\mu\text{g/ml}$ responded to therapy. Our results showed that the in vitro break point for clinical resistance is probably higher than presently recommended. The few clinical failures with spectinomycin were probably due to reinfections or patients' immunological differences.

Recently, Boslego and coworkers²⁶ reported the emergence of a substantial prevalence of clinical treatment failures caused by spectinomycin-resistant strains (MIC $\geq 100 \mu\text{g/ml}$) in a geographic region where spectinomycin had been used in the primary treatment of gonococcal infection for only three years. Since the completion of the clinical study, we initiated a surveillance program to monitor the emergence of

resistant strains to spectinomycin using sensitivity testing with 100 μg -spectinomycin disk and agar dilution. We have documented only a few sporadic (2) cases in a four year period, but a cautious approach to the widespread use of spectinomycin is recommended. Public health officials in the area must remain vigilant in their surveillance effort and be alert to the introduction or emergence of spectinomycin-resistant strain.

There were no serious adverse reactions found with either the APPG plus probenecid or spectinomycin regimens. APPG plus probenecid could be used in Puerto Rico only in clinics that have surveillance for PPNG and CMRNG strains. However, spectinomycin could be given as an alternative for the treatment of genital gonorrhoea because of the high prevalence of CMRNG and PPNG strains and the high rate of failure seen with the use of penicillin. Current recommendations include a course of tetracycline or erythromycin for non-gonococcal urethritis (dual therapy) added to spectinomycin (or ceftriaxone) for genital gonorrhoea.²⁷

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